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Effects of 28 day exposure to cold temperature or feed restriction on growth, body composition, and expression of genes related to muscle growth and metabolism in channel catfish

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Abstract

Cold temperature decreases feed intake and growth of channel catfish, but the physiological and molecular mechanisms associated with the growth depression remain unknown. Therefore, an experiment was conducted to determine the effects of a 28-day exposure to cold temperature or feed deprivation at warm temperature on growth, physiological indices, and the expression of genes involved in muscle growth and metabolism. Juvenile channel catfish (initial mean weight 119.7 ± 8.0 g) were stocked into six 189-l tanks (20 fish per tank) with two replicate tanks for each of three treatments: Cold-fish reared at ≈ 10 °C and fed to excess once daily, Feed-Restricted-fish reared at ≈ 27 °C and deprived of food, and Control-fish reared at ≈ 27 °C and fed to excess once daily. Muscle and blood samples were collected on days 1, 14, and 28, and final body weight, organ weight, and fillet fat and moisture were collected on day 28. Plasma samples were analyzed for cortisol and creatine-kinase activity. Abundance of mRNAs encoding myostatin, myosin heavy chain (MHC), and heat shock protein-70 (HSP-70) in muscle was determined via real-time quantitative PCR. Final body weight, weight gain, specific growth rate, condition factor, and liposomatic index were highest in control fish, intermediate in cold treatment fish, and lowest in feed-deprived fish. Fillet fat was lower and fillet moisture was higher for feed-restricted fish than for cold and control fish. Cold treatment fish had a greater hepatosomatic index than either control or feed-deprived fish. On day 1 cold treatment fish had greater plasma cortisol concentrations than control fish. Creatine kinase activity was greater for cold and feed-deprived fish than for control fish on day 14. Myostatin mRNA abundance was lower for cold and feed-deprived treatment fish than for control fish on day 1. Myostatin and HSP-70 mRNA was increased on day 14

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and day 28 in the cold treatment fish relative to the control or feed-deprived fish. MHC mRNA was increased in the cold treatment fish on day 14. Voluntary suppression of feed intake associated with cold water temperature and feed restriction at warm temperature both reduced growth in channel catfish, but patterns of gene expression in the two treatments were not similar.

Keywords: Temperature effects; Myostatin; Myosin; Fasting; Catfish

1. Introduction

Farming of channel catfish is the largest sector of the U.S. aquaculture industry with approximately 270 million kg of catfish processed in 2001 (USDA, 2002). Increasing the rate of gain in lean muscle mass is a common goal in meat animal production, including channel catfish farming. However, unlike traditional livestock species, feeding rates for farm-raised catfish vary greatly throughout the production cycle and these changes in feeding rates dramatically affect growth. Channel catfish are typically fed to satiation once daily during the feeding season (May through October in the southeastern U.S.; Tucker and Robinson, 1990), but fish are fed infrequently or not at all during the winter when cold water temperatures suppress voluntary feed intake. In addition, cessation of feeding is a commonly used management practice during the normal feeding season to minimize mortalities during outbreaks of Enteric Septicemia of Catfish, a bacterial disease responsible for up to 50% of total annual catfish mortalities (Hawke et al., 1998). Although the reduction in growth associated with feed restriction has been documented in channel catfish (Gaylord and Gatlin, 2000; Peterson and Small, 2004), the underlying physiological responses and changes in gene expression associated with the growth depression due to feed restriction are not well known.

The expression of several genes relative to the accretion of skeletal muscle is presently of considerable interest. In mammals, myostatin (MSTN) negatively regulates muscle growth by inhibiting myoblast proliferation (Thomas et al., 2000) and differentiation (Langley et al., 2002). Glucocorticoids have been shown to upregulate MSTN expression in mammals (Ma et al., 2001, 2003) and downregulate MSTN in fish (Rodgers et al., 2003). Exposure to cold temperature has been found to increase plasma cortisol in tilapia (Chen et al., 2002). Therefore, it is plausible that lowering the water temperature alters the abundance of MSTN in fish via a glucocorticoid mediated mechanism. The expression of proliferating cell nuclear antigen (PCNA) in muscle is increased in response to feeding in mammals (Jeanplong et al., 2003) and in the Antarctic fish *Notothenia coriiceps* (Brodeur et al., 2002). Therefore, it is possible that PCNA expression in channel catfish is altered by low temperatures, either due to or independent of feeding status. Upregulation of heat-shock protein-70 (HSP-70) in mammalian skeletal muscle is suggested to play a role in preventing muscle damage or atrophy in response to environmental stressors (Oishi et al., 2003). The abundance of heat shock protein-70 (HSP-70) is rapidly increased in brain tissue of channel catfish in response to cold acclimation (Ju et al., 2002). However, no studies have addressed HSP-70 mRNA expression in the skeletal muscle of channel catfish. The expression of various isoforms of myosin heavy chain (MHC) is increased in response to cold temperature in several warmwater fish species (Johnston, 2001), but little is known about the affect of low water temperature on the expression of MHC in the channel catfish. The expression level of myosin mRNA has been shown to be associated with increased specific growth rates in rainbow trout *Oncorhynchus mykiss* (Overturf and Hardy, 2001). This contradicts the increase in MHC found in warmwater fish species in response to cold temperature, as lowering the water temperature decreases specific growth rate. In the present study, we sought to establish the impact of prolonged exposure to cold water temperature or restricted feeding at warm temperatures on growth of channel catfish, and

relate this effect to changes in the expression of genes linked to muscle growth.

2. Materials and methods

2.1. Research animals and sampling

The experimental protocol was approved by the Animal Care and Use Committee, National Warmwater Aquaculture Center. The fish used in these experiments were a channel catfish strain (NWAC103) maintained by the National Warmwater Aquaculture Center, and reared at the USDA-ARS Catfish Genetics Research Unit aquaculture facility located at Stoneville, MS. Juvenile fish ($n=120$; Average body weight 119.7 ± 8.0 g) were randomly stocked (stratified by family of origin) into six 189-l tanks at a density of 20 fish per tank. Each fish was tagged with a passive integrated transponder (BioMark Boise, ID) to allow individual identification. The fish were reared under a 14:10 h light/dark photoperiod. After an acclimation period of 2 weeks at a water temperature of approximately 25 °C, the water temperature in two of the tanks was decreased and maintained at an average temperature of 10.5 ± 0.3 °C for 28 days. Water temperatures were lowered over a 48 h period. The fish in two of the remaining tanks were subjected to 28 days of complete food deprivation. The fish in the tanks that were chilled and the control group of fish in the remaining two tanks were fed a commercial floating catfish feed (36% crude protein; Land O'Lakes Farmland Feed LLC, Fort Dodge, IA) once daily to excess. The average water temperature in the tanks containing the control and food-deprived fish was 24.9 ± 0.5 °C. At sampling times, the fish (3 fish/tank) were euthanized with an overdose (200 ppm) of tricainemethane sulfonate (Finquil; Argent Chemical Laboratories, Redmond, WA, USA). The fish were bled from the caudal vasculature into heparin-coated syringes, and the resulting plasma was separated and frozen at −80 °C. Muscle samples from the dorsal white musculature were collected, snap frozen in liquid nitrogen, and stored at −80 °C until total RNA was isolated. On day 28 of the experiment, body weight and body length were measured, and the liver and mesenteric body fat were removed and weighed from all remaining fish.

2.2. Calculation of specific growth rate, condition factor, hepato-, and liposomatic index

Specific growth rates (SGR) were calculated using the formula $((\ln(\mathrm{WT}) - \ln(\mathrm{wt}))T^{-1}) \times 100$ where WT and wt are final and initial weights, respectively, and T is the time in days. Fish condition factor was calculated by dividing the body weight by the body length^3 and multiplying by 100. The hepatosomatic index was calculated by dividing liver weight by body weight and multiplying by 100. Likewise, the liposomatic index was determined by dividing mesenteric fat weight by body weight and multiplying by 100.

2.3. Fillet proximate composition determination

The left-side fillet was removed from three fish per tank (6 fish per treatment), ground to a paste using a food processor, and frozen at −20 °C for subsequent analysis. Crude fat (ether extraction) and moisture (oven drying) of fillet samples were determined using methods described by the AOAC (2000).

2.4. Plasma cortisol and creatine kinase activity determination

Plasma concentrations of cortisol were measured using a DELFIA® time-resolved fluroimmoassay kit (Perkin-Elmer Life Sciences, Boston, MA). This assay has been previously validated for the quantification of plasma cortisol in channel catfish (Small and Davis, 2002). Plasma creatine kinase activity was measured using a commercial kit (Pointe Scientific Lincoln Park, Michigan) per the manufacturer's instructions.

2.5. RNA isolation and cDNA synthesis

Total RNA was isolated using TRI-reagent® (Molecular Research Center, Cincinnati, OH) according to the manufacturer's protocol, and the RNA pellets were resuspended in TE-8 (Tris–EDTA, pH 8.0). To eliminate possible genomic DNA contamination, the RNA samples were treated with a DNase I kit (DNA-free, Ambion, Austin, TX) per the manufacturer's instructions. The total RNA was quantified by measuring the absorbance at 260 nm using a NanoDrop® ND-100 spectrophotometer (NanoDrop Technologies, Rockland, DE), and the purity was

assessed by determining the ratio of the absorbance at 260 and 280 nm (NanoDrop). All samples had 260/280 nm ratios above 1.8. Additionally, the integrity of the RNA preparations was verified by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 1.0% agarose gels. Total RNA (1 μg) was reverse transcribed using a commercially available cDNA synthesis kit (iScript, BioRad Laboratories Hercules CA).

2.6. Quantification of mRNA expression by quantitative RT-PCR

The sequences of the primers and probes used to quantify the cDNAs for MSTN, MHC, HSP-70, and PCNA are shown in Table 1. The reporter dye used for each probe was 6-FAM. Amplification was carried out in a total volume of 25 μL containing 1X supermix (Bio-Rad), forward and reverse primers (100 nM; Table 1) dual-labeled probe (100 nM) and 1 μL of the cDNA reaction. The PCR reactions were cycled 40 times using a Bio-Rad iCycler iQ Real-Time PCR detection system (Bio-Rad). A nontemplate control was run with every assay, and all determinations were performed in duplicate. External cDNA standards, ranging from 10^9–10^2 molecules, were constructed by cloning the corresponding RT-PCR product into a pCR® 4-TOPO® vector (Invitrogen, Carlsbad, CA). The identities of the cloned inserts were confirmed by sequencing at the USDA Mid-South-Area Genomics Laboratory. The concentration (μg/μL) of the standards was determined by measuring the absorbance of the plasmid preparation at 260 nm. The copy number of plasmids per μL (CN) was calculated with the following formula: $CN = 9.1 \times 10^{11}$ (concentration of the plasmid/size of the plasmid in Kb). The number of templates present in the experimental reactions is calculated by the Bio-Rad iCycler iQ system software (Bio-Rad) based on the standard curve data. Using the

Table 2
Effect of cold temperature and food deprivation on growth characteristics, plasma cortisol, and creatine kinase activity in channel catfish

Item	Control	Cold	Feed-deprived	SE	*P*-value
Body weight (g)	245.6^{x}	137.8^{y}	97.1^{z}	10.5	0.0001
Weight gain (g)	115.9^{x}	13.7^{y}	-8.0^{z}	4.4	0.0001
Length (mm)	296.4^{x}	256.3^{y}	243.4^{y}	5.5	0.0001
Specific growth rate	1.9^{x}	0.3^{y}	-0.2^{z}	0.04	0.0001
Condition factor	0.9^{x}	0.8^{y}	0.7^{z}	0.02	0.0001
Hepatosomatic index	1.4^{x}	2.7^{y}	0.6^{z}	0.07	0.0001
Liposomatic index	1.8^{x}	0.9^{y}	0.3^{z}	0.08	0.0001
Fillet fat	5.2^{x}	4.3^{x}	2.8^{y}	0.5	0.01
Fillet moisture	75.0^{x}	$75.8^{x,y}$	77.3^{y}	0.7	0.08
Cortisol (ng/mL)					
day 1	2.7^{x}	6.5^{y}	$4.2^{x,y}$	1.1	0.08
day 14	2.7	7.8	6.0	2.1	0.24
day 28	2.7	2.8	2.7	0.1	0.39
Creatine kinase (IU/L)					
day 1	612.0	108.8	679.0	213.0	0.15
day 14	418.6^{x}	1415.6^{y}	1197.6^{y}	273.0	0.05
day 28	596.0	301.0	448.8	201.6	0.60

^{x,y}Different superscript letters denote significant differences within each row ($P < 0.05$).

Table 1
Primer and internal probe sequences used for quantitative PCR

Gene	Primer sequences[a]	Accession number
Myostatin	(S) CTCGGGGACGACGGCAAG	AF396747
	(AS) CTTGAACGTCGGGGTTGG	
	(P) CACCGAGACCGTCATGAGCATGGCC	
PCNA[b]	(S) ACCTCAGCAGTATGTCCAAG	AY566306
	(AS) CAGAGAGTCTGCATTGTCCT	
	(P) TGTGCTGGGGAATGAGGACATCA	
HSP-70[b]	(S) CTGCTGGTGACACTCATCTT	U22460
	(AS) CGAACAGCTCTCTTGTTGTC	
	(P) CGCAAGCACAAGAAGGACATCAGT	
Myosin	(S) TGATGACCCACCTCAGTGAA	AY728019
	(AS) CACAGTGACGCAGAACAACC	
	(P) AAGAGCGTTACGCAGCATGGATGAT	

[a] S = sense primer, AS = antisense primer, P = oligonucleotide probe.
[b] PCNA = proliferating cell nucleus antigen; HSP-70 = heat shock protein-70.

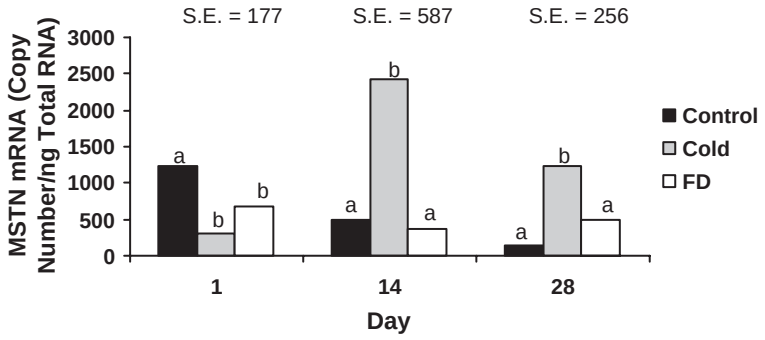

Fig. 1. Effect of cold temperature and food deprivation (FD) on the abundance of myostatin (MSTN) mRNA in the muscle of channel catfish. Muscle samples were collected from the fish on day 1, day 14, and day 28 of the experiment. The values presented are means for 6 fish per treatment at each time point. Within a time point, treatment means with different letters are significantly different ($P<0.05$).

amounts of cDNA reverse-transcribed from a known amount of total RNA, the number of specific molecules of mRNA/nanogram total RNA can be calculated (Bustin, 2002).

2.7. Statistical analysis

The statistical analyses were conducted as one-way ANOVAs using the Statistical Analysis System Version 8.0 software (SAS Institute, Cary, NC). Separate ANOVAs were conducted for each sampling time point. When ANOVA indicated a significant difference ($P<0.10$), the means were separated using the Student–Newman–Keuls multiple range test. A significance level of $P<0.05$ was used for mean separation. For each variable measured, the individual fish served as the experimental unit. Data are presented as means ± standard error (S.E.).

3. Results

Fish that were exposed to cold temperature or fish that were deprived of feed weighed less than control fish at the completion of the experiment (Table 2). The body weight gain of fish reared at cold temperature was 89% less than the control fish over the course of the experiment, whereas the feed-deprived fish lost body weight. This is reflected in the lower specific growth rates of the cold-exposed fish as compared to control fish, and the negative specific growth rates of the feed-deprived fish. Body length was affected by treatment as both cold-exposed and feed-deprived fish had lower body lengths compared to the control fish. The condition factor and liposomatic index were highest for control fish, intermediate in cold treatment fish, and lowest in feed-deprived fish. The hepatosomatic index was highest in cold treatment fish,

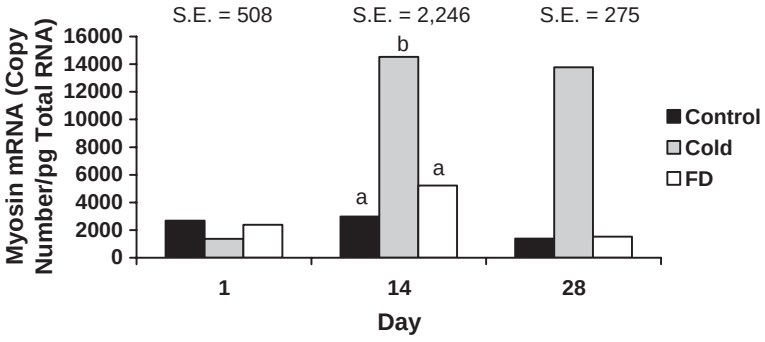

Fig. 2. Effect of cold temperature and food deprivation (FD) on the abundance of myosin heavy chain (MHC) mRNA in the muscle of channel catfish. Muscle samples were collected from the fish on day 1, day 14, and day 28 of the experiment. The values presented are means for 6 fish per treatment at each time point. Within a time point, treatment means with different letters are significantly different ($P<0.05$).

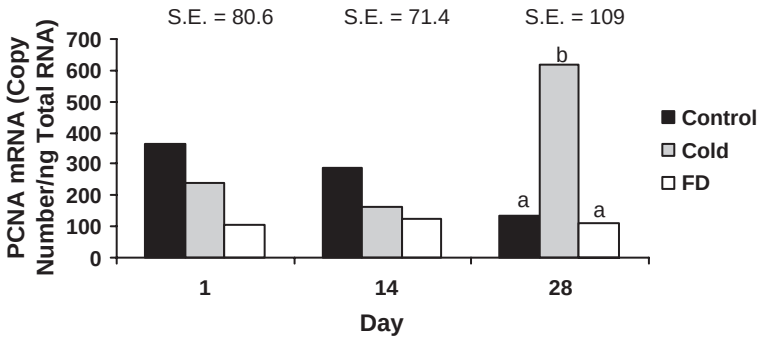

Fig. 3. Effect of cold temperature and food deprivation (FD) on the abundance of proliferating cell nuclear antigen (PCNA) mRNA in the muscle of channel catfish. Muscle samples were collected from the fish on day 1, day 14, and day 28 of the experiment. The values presented are means for 6 fish per treatment at each time point. Within a time point, treatment means with different letters are significantly different ($P<0.05$).

intermediate in control fish, and lowest in feed-deprived fish. Fish reared at the cold temperature had greater plasma cortisol concentrations than control fish on day 1 of the experiment. However, there were no differences in plasma cortisol concentrations between treatment groups on days 14 or 28. Plasma creatine kinase activity was elevated in fish reared at the cold temperature and in feed-deprived fish on day 14 of the experiment. Creatine kinase activity was not affected by treatment on day 1 or day 28.

On day 1 of the study, the abundance of MSTN mRNA was decreased in cold treatment and feed-deprived fish (Fig. 1). The abundance of MSTN mRNA was found to be elevated in the muscle tissue of fish reared at cold temperature when compared to either control or feed-deprived fish on day 14 and day 28. The abundance of an mRNA encoding MHC was increased in cold-exposed fish on day 14 (Fig. 2). Likewise, the abundance of PCNA mRNA in muscle tissue was increased (Fig. 3) in fish reared at cold temperature on day 28. Fish reared at the cold temperature had an increased abundance of muscle HSP-70 mRNA on day 14 and 28 compared to other treatments (Fig. 4).

4. Discussion

Our findings substantiate the results of previous studies (Buentello et al., 2000; Silverstein et al., 2000) in which low water temperatures were found to decrease the specific growth rate of channel catfish. Cold temperature elicited a modest increase in plasma cortisol levels on day 1 of this study. This finding is in conflict with the lack of a cortisol response due to low water temperature noted by Davis et al. (2001), but agrees with the increase in plasma cortisol found in carp in response to cold-stress. We did not find an

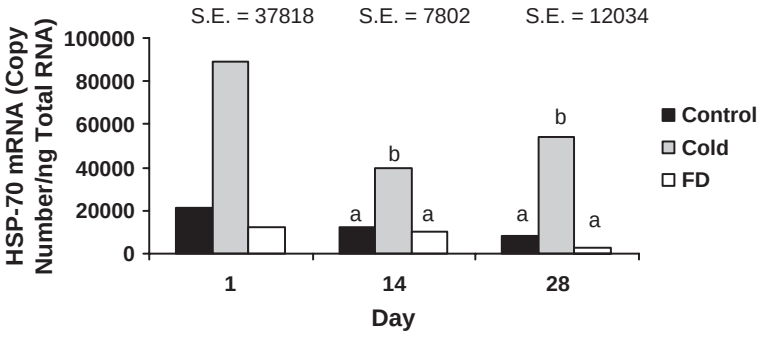

Fig. 4. Effect of cold temperature and food deprivation (FD) on the abundance of heat-shock protein-70 (HSP-70) mRNA in the muscle of channel catfish. Muscle samples were collected from the fish on day 1, day 14, and day 28 of the experiment. The values presented are means for 6 fish per treatment at each time point. Within a time point, treatment means with different letters are significantly different ($P<0.05$).

increase in plasma cortisol in fish that were deprived of feed for 14 or 28 days. This finding disagrees with what was found in a recent study in which cortisol concentrations were elevated in channel catfish that were fasted for 30 days (Peterson and Small, 2004). The lack of a difference in plasma cortisol response may be due to time of sampling or other factors. Furthermore, it appears as if plasma cortisol concentrations are transiently increased in response to prolonged fasting, as Peterson and Small (2004) found that cortisol was elevated on day 30, but not days 14, 45, or 60 of a 60 day fast. Nonetheless, the increased cortisol concentrations found in the fish reared at cold temperature coincided with a decreased abundance of MSTN mRNA on day 1 of the experiment. This agrees with our previous finding that an elevation of glucocorticoids is associated with a decreased abundance of MSTN mRNA (Weber et al., 2005). Interestingly, MSTN mRNA abundance was decreased in the feed-deprived fish on day 1 even though the plasma cortisol levels of the feed-deprived fish did not differ from the other two treatment groups. However, it is possible that we missed the peak in the cortisol response due to sampling time. Further research is necessary to determine whether the long term elevation in MSTN mRNA found in catfish reared at cold temperatures is mediated by glucocorticoids.

In the present study we found that prolonged exposure to low water temperature increases the abundance of MSTN mRNA in muscle tissue. This is the first report of MSTN expression being regulated by temperature in any aquatic species. The ability of cold temperature to increase MSTN mRNA abundance appears to be independent of a substantial decrease in feed intake as the fasted controls were not found to have elevated MSTN mRNA levels. The finding that prolonged fasting has no effect on channel catfish MSTN mRNA abundance differs from what was found in tilapia larvae (Rodgers et al., 2003). Rodgers et al. (2003) found that fasting for a period of 9 days decreased the abundance of whole body MSTN mRNA. However, our data do agree with the findings of Chauvigne et al. (2003) in which long-term feeding status had no effect on MSTN mRNA abundance in rainbow trout muscle. The different findings may be partially due to differences in sampling times, or that we analyzed muscle tissue, and not whole body MSTN mRNA abundance. Indeed, it has been found that MSTN mRNA transcripts are present in several tissues in addition to skeletal muscle in channel catfish (Kocabas et al., 2002) and in other species of fish (Ostbye et al., 2001; Rodgers et al., 2001).

We concede that the observed changes in mRNA may not have resulted in changes in protein levels under these circumstances. However, we were primarily interested in the regulation of MSTN at the genetic level in order to compare with what has been noted in other warmwater fish species in which the fish were subjected to scenarios which depress growth (Rodgers et al., 2003; Vianello et al., 2003). Nonetheless, these data indicate regulation of MSTN at the genetic level by cold temperatures in channel catfish. Other studies which have investigated the regulation of myostatin expression in fish by glucocorticoids and feeding status (Chauvigne et al., 2003; Rodgers et al., 2003) also fail to address the question of protein abundance, likely due to the lack of available antibodies. Our laboratory is currently attempting to acquire and screen antibodies that might be useful for these MSTN and other proteins in catfish, but have not had success with them at this point.

Whether the elevated level of MSTN mRNA we found in fish reared at low temperature is associated with muscle protein catabolism remains to be determined. Indeed, elevated levels of plasma creatine kinase activity were found in both cold-exposed and fasted fish on day 14 of the experiment. Elevations in plasma creatine kinase activity are associated with muscle degeneration in Atlantic salmon (Ferguson et al., 1986). However, as previously mentioned MSTN mRNA levels were not elevated in the fasted fish. This leads us to speculate that elevations in MSTN mRNA are not necessarily associated with protein catabolism. It is interesting that we found both an increase in MSTN mRNA and a corresponding increase in MHC mRNA in the fish reared at cold temperatures on day 14. This would seem counter-intuitive given that MSTN has been shown to be a negative regulator of myoblast differentiation in mammalian models. However, previous research in carp has shown that prolonged exposure to cold temperatures leads to an increased transcription of the gene encoding a MHC isoform with a greater ATPase activity (Johnston, 2001). We also found increases in

both MSTN and PCNA mRNAs in muscle tissue on day 28 of the experiment. Again, this contradicts the role of MSTN mRNA as a negative regulator of myoblast proliferation as PCNA is a cofactor to DNA polymerase and its abundance is positively correlated with DNA synthesis (Bravo et al., 1987; Baserga, 1991). In addition, increased abundance of PCNA mRNA in skeletal muscle has been shown to be associated with rapid muscle growth in sheep (Jeanplong et al., 2003). The increased PCNA mRNA abundance found in muscle tissue of cold-exposed fish may be indicative of a mechanism to counteract the muscle catabolism due to skeletal muscle remodeling. Another possibility is that the PCNA mRNA is increased in several different cell types in addition to myoblasts because muscle tissue contains other cell types such as adipocytes and fibroblasts. This may reflect a compositional change taking place in muscle tissue in response to low water temperature. Nonetheless, further research is necessary to characterize the roles of MSTN and PCNA in fish muscle growth and development.

An increased expression of HSP-70 in brain tissue has been previously linked to cold acclimation in channel catfish (Ju et al., 2002). The results of our experiment extend their finding to include muscle tissue. The abundance of HSP-70 mRNA was increased in the fish reared at cold temperature on days 14 and 28 of the study. This data agrees with what has been found in rat cardiomyocytes (Laios et al., 1997) and in the muscle of carp (Ali et al., 2003) where cold shock has been found to increase the expression of HSP-70. The role of HSP-70 in muscle metabolism is not well understood, but upregulation of HSP-70 has been associated with increases in muscle mass due to overloading in the rat (Locke et al., 1994), muscle protein repair or protection (Kilgore et al., 1998), and MHC fiber type changes (Locke et al., 1991). The biological ramifications of increased HSP-70 in muscle tissue remains to be determined and further research is necessary to characterize the role of HSP-70 in fish muscle.

It appears as if feed deprivation puts a greater demand on body energy stores than does cold water temperature as the liposomatic index and condition factor were decreased to a greater extent in the feed-deprived fish than fish reared at cold temperatures. As previous investigations have found (Gaylord and Gatlin, 2000), the decreased demand on energy stores is reflected in the greater fillet fat content in fish reared at cold temperature as compared to the feed-restricted fish. This is an interesting finding given that the feed intake observed in the cold-exposed fish was comparable to that of the fish that were deprived of feed. Cold temperature fish were offered feed to excess to insure they had adequate opportunity to feed, therefore it was not possible to accurately measure feed intake. However, observation of feeding activity indicated that feed intake was greatly reduced in cold temperature fish compared to controls. The increase in energetic efficiency at low temperatures may be reflected by the increase in the hepatosomatic index found in fish reared at cold temperatures. Previous investigations have found increased total protein content (Kent et al., 1988) and enzyme activities (Seddon, 1997) in channel catfish subjected to low water temperatures. Indeed, the increase in liver size which is reflective of an increased abundance of enzymes (Kent et al., 1988), may serve as an adaptive response in order to compensate for a decrease in enzymatic activity that is found at lower temperatures.

In summary, channel catfish reared at a low temperature grow slower and have an increased abundance of MSTN mRNA in muscle tissue than do control fish or fasted fish. Rearing fish at cold temperature increased the expression of HSP-70 in the muscle tissue. Paradoxically, the elevated levels of MSTN expression were not associated with a decreased expression of myosin or PCNA in muscle tissue. The decreased growth rate of the fasted fish was independent of changes in the expression of MSTN, myosin heavy chain, or PCNA.

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